Paroxetine Inhibits Acute Effects of 3,4-Methylenedioxymethamphetamine on the Immune System in Humans

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ABSTRACT

The effect of pretreatment with paroxetine on cell-mediated immune response and release of cytokines after the administration of 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) was investigated in a double-blind, randomized, crossover, controlled clinical trial in which 12 healthy male recreational users of MDMA participated. Subjects received 20 mg/day paroxetine (or placebo) for the 3 days before MDMA challenge (100 mg). Acute MDMA administration produced a time-dependent decrease in CD4 T-helper cells, a decrease in the functional responsiveness of lymphocytes to mitogenic stimulation, a simultaneous increase in natural killer (NK) cells as well as cortisol and prolactin stimulation kinetics. A high increase in the release of anti-inflammatory cytokines (transforming growth factor-β and interleukin-10) with a simultaneous decrease of anti-inflammatory response (interleukin-2) was also observed. Pretreatment with paroxetine partially reduced MDMA effects on CD4 T and NK cells, whereas totally inhibiting the suppression of the immune response to mitogens and alterations in cytokines release. MDMA-induced alterations in the immune system as well as antagonistic effects mediated by paroxetine show a trend toward baseline levels at 24 h. These findings suggest that acute effects of MDMA on immune system are mainly mediated by its interaction with the serotonin transporter and subsequent serotonin release with a possible participation of other neuroendocrine regulatory systems.

There is evidence that 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) exerts immunomodulating properties in humans. Previous studies (Pacifici et al., 1999, 2000, 2001a,b, 2002) have shown that a single dose of 100 mg of MDMA produced a decrease in CD4 T-helper cells with a simultaneous increase in natural killer (NK) cells, and a decrease in functional responsiveness of lymphocytes to mitogenic stimulation, whereas total leukocyte count remained unchanged. Acute MDMA treatment also produced a high increase of immunosuppressive cytokines [transforming growth factor (TGF)β1 and interleukin (IL)-10], and a switch from Th1-type cytokines (IL-2 and interferon-γ) to Th2-type cytokines (IL-4 and IL-10). The correlation of MDMA pharmacokinetics and MDMA-induced cortisol stimulation kinetics with the profile of MDMA-induced immune dysregulation suggested an implication of central monoaminergic system with the release of corticotrophin-releasing factor and subsequent activation of the hypothalamic-pituitary-adrenal axis. Immune function was almost completely restored at 24 h from acute administration. By contrast, after chronic consumption of MDMA, absolute number of lymphocytes and in particular T lymphocytes and CD4 T-helper cells subsets showed a trend toward reduced values, although within normal range values, whereas NK cells were reduced to one-third of those from healthy individuals with a statistically significant decrease in affected immune parameters recorded during a 2-year observation period (Pacifici et al., 2002). However, the neurochemical mechanisms underlying the im-

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ABBREVIATIONS: MDMA, 3,4-methylenedioxymethamphetamine; NK, natural killer; TGF, transforming growth factor; IL, interleukin; Th, T-helper; 5-HT, 5-hydroxytryptamine; SSRI, selective serotonin reuptake inhibitor; PBMC, peripheral blood mononuclear cell; PHA, phytohemagglutinin A; ConA, concanavalin A; SI, stimulation index; PE, phycoerythrin; FITC, fluorescein isothiocyanate; AUC, area under the curve; DA, dopamine; HMMA, 4-hydroxy-3-methoxymethamphetamine.
munological effects of MDMA have not been clearly elucidated.

In contrast, the pharmacology of MDMA has been well characterized in animal models and in humans (Cami et al., 2000; de la Torre et al., 2000; Green et al., 2003). MDMA releases serotonin (5-hydroxytryptamine, 5-HT) mainly through an interaction with the serotonin uptake site. In animals, selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine and citalopram, were found to block the MDMA-induced serotonin release (Hekmatpanah and Peroutka, 1990; Berger et al., 1992; Gudelsky and Nash, 1996). In addition, fluoxetine prevents the long-term toxic effects of MDMA on serotonin neurons (Malberg et al., 1996). In humans, the administration of citalopram was shown to reduce psychological, cardiovascular, and vegetative effects of MDMA in healthy volunteers, suggesting that these effects could be mediated by the serotonin transporter and subsequent serotonin release (Liechti et al., 2000; Liechti and Vollenweider, 2000). In clinical trials, MDMA has also been shown to release dopamine and norepinephrine through an effect on the membrane reuptake transporter of these neurotransmitters (Liechti and Vollenweider, 2001). Moreover, MDMA acts as a direct agonist at the serotonin 5-HT-2, α-2-adrenergic, and muscarinic M-1 receptor levels. According to recent reports, the presence of neurotransmitter transporters in human lymphocytes further supports the involvement of neurotransmitters in the regulation of immune function (Marazziti et al., 1998; Mössner and Lesch, 1998; Mizruchin et al., 1999; Basu and Dasgupta, 2000).

In a rat model, Connor et al. (2000) suggested the possible role of dopamine in the immunosuppressive effects of MDMA. Indeed, they showed that the suppressive effect of MDMA on lipopolysaccharide-induced proinflammatory cytokine secretion cannot be attributed exclusively to the serotonin-releasing properties because pretreatment with paroxetine did not alter MDMA-induced effects on interleukin-1β and tumor necrosis factor-α. Paroxetine, (3S,4R)-4-[4-(4-fluorophenyl)-3-(3,4-methylenedioxy phenoxymethyl)piperidine], is a well known SSRI, used worldwide in therapeutics as antidepressant drug. Paroxetine lacks intrinsic immunogeneity (Henderson et al., 1988), has a nonlinear pharmacokinetics, and may interfere with the metabolism of other drugs by inhibiting CYP2D6, a polymorphic member of the cytochrome P450 superfamily of enzymes. Paroxetine produces, both in vitro and in vivo, a catalytically inactive complex with CYP2D6 as MDMA does (Delaforge et al., 1999; Bertelsen et al., 2003). On the other hand, the effects of CYP2D6 inhibition by paroxetine on pharmacokinetics and metabolism of MDMA in humans have been recently reported (Farré et al., 2002). It was shown that pretreatment with paroxetine significantly increased systemic disposition of MDMA by a 20 to 30% compared with placebo/MDMA treatment but decreased the cardiovascular and subjective effects of MDMA. Hence, a double action of paroxetine, an inhibition of both MDMA-induced serotonin release and MDMA metabolism, may be anticipated to interfere on the immunomodulating properties of MDMA.

The present study was therefore undertaken to investigate the effect of pretreatment with paroxetine on cell-mediated immune effects of MDMA in 12 healthy subjects who participated in a double-blind placebo-controlled study.

Materials and Methods

Subjects

Male subjects were recruited by word of mouth. Eligibility criteria required the recreational use of MDMA on at least five occasions. Exclusion criteria included daily consumption of more than 20 cigarettes and more than 30 g of ethanol (3 units/day). Eligible subjects were interviewed by a psychiatrist (structured clinical interview for Diagnostic and Statistical Manual-Version IV) to exclude the presence of major psychiatric disorders, including schizophrenia, psychosis, and major affective disorder. Each participant underwent a general physical examination, routine laboratory tests, urinalysis, and a 12-lead electrocardiogram to confirm his or her health status.

Twelve subjects gave written consent to participate in the study and were economically compensated for the possible inconveniences derived from the procedures. The study was conducted in accordance with the Declaration of Helsinki, approved by the Ethical Committee of our institution, and authorized by the Spanish Ministry of Health.

Demographic and anthropometric characteristics of the study population included a mean age of 24 (range 19–34) years, mean weight of 71.0 (range 65.5–84.0) kg, and mean height of 177.0 (167.5–190) cm. Participants were both current smokers (n = 4) and nonsmokers (n = 8), and their average consumption of alcohol was 10 units/week. All of them had previous experience with the consumption of cannabis, cocaine, and methamphetamine. None had history of abuse or drug dependence according to Diagnostic and Statistical Manual-Version IV criteria (except for nicotine dependence), nor had experienced any medical or psychiatric adverse reaction after MDMA consumption. All participants were classified as extensive metabolizers for CYP2D6 using dextromethorphan as a drug probe (Schmidt et al., 1985).

Study Design

The study design was double-blind, randomized, crossover, and controlled. Treatment conditions (paroxetine/MDMA and placebo/MDMA) were randomly assigned. Volunteers were requested to abstain from consumption of any drug of abuse during the study period, and urine drug testing was performed before each experimental session for opioids, cocaine, cannabis, and amphetamines. For all four groups of substances tested, all volunteers were negative before each experimental session. Each subject participated in two, 3-day study sessions, with a washout period of 15 days. In each session, subjects arrived at the laboratory at 8:00 AM after an overnight fast and had an indwelling intravenous catheter inserted into a subcutaneous vein in the forearm of the non-dominant arm. Thereafter, they remained seated in a quiet room throughout the session. Subjects randomized to the paroxetine/MDMA condition received paroxetine (20 mg/day on days 1, 2, and 3) and MDMA (100 mg on day 3), whereas those randomized to the placebo/MDMA condition were given placebo (on days 1, 2, and 3) and MDMA (100 mg on day 3). The doses of paroxetine or placebo doses were administered around 9:00 AM in fasting conditions. Taking into account the average T_max of MDMA and paroxetine (2 h for MDMA and 5 h for paroxetine) (Mas et al., 1999; Segura et al., 2003), MDMA was administered 3 h later than the last dose of paroxetine to obtain maximum plasma concentrations of both drugs at the same time (12:00 AM). On day 1, blood samples were collected at baseline (0 h) and at 3, 8, and 24 h after the administration of paroxetine or placebo (immediately before the second dose of paroxetine or placebo). On day 3, blood samples were drawn before the third dose of paroxetine or placebo (48 h after the first administration) and at 3, 4.5, 8, 11, and 24 h after paroxetine or placebo administration (that represented 0 h, 1.5, 5, 8, and 21 h after MDMA administration). Blood samples for pharmacokinetic studies and analysis of hormones were collected in heparinized tubes (3, 4, 5, 6, 7, and 9 h after paroxetine or placebo administration). Cardiovascular effects were monitored and questionnaires for assessing the subjective effects were administered during the experimental sessions at various intervals (data not shown).
The doses of paroxetine and MDMA as well as the schedule of blood sampling were chosen according to data of previous studies (Mas et al., 1999; Segura et al., 2003). Paroxetine was supplied as Seroxat (GlaxoSmithKline, Tres Cantos, Madrid, Spain) and prepared, similarly to placebo, by the Service of Pharmacy of Hospital del Mar (Barcelona, Spain) as white, soft gelatin capsules. MDMA was supplied by the Spanish Ministry of Health and prepared by Service of Pharmacy as soft gelatin capsules.

**Determination of Plasma Cortisol and Prolactin**

Plasma cortisol concentrations were determined by fluorescence polarization immunoassay (Abbott Diagnostics, Chicago, IL) according to the manufacturer’s instructions. The intra-assay coefficients of variation were 2.9 and 2.6 for low (4.0 μg/dl) and high (40.0 μg/dl) controls, respectively. The assay sensitivity was 0.45 μg/dl. Plasma prolactin concentrations were measured with a microparticles enzyme immunoassay (AXSYM; Abbott Diagnostics) according to the manufacturer’s instructions. The intra-assay coefficients of variation were lower than 4.2 and 3.5% for low (8.0 ng/ml) and high (40.0 ng/ml) controls, respectively. The assay sensitivity was 0.60 ng/ml.

**Blood Cell Preparation for Immunological Tests**

Peripheral blood was collected in evacuated tubes containing ethylenediaminetetraacetic acid (0.47 M). Complete blood cell count and biochemical profile were obtained for each participant. Peripheral blood mononuclear cells (PBMCs) were obtained by centrifugation of the whole blood on a Ficoll-Hipaque density gradient. PBMCs were rinsed and suspended in tissue culture (RPMI 1640 medium) containing penicillin (100 U/ml), streptomycin (100 μg/ml), and 10% fetal bovine serum.

**Response to Mitogens.** PBMC samples were adjusted to a final concentration of 1 × 10^7 cells/ml, placed in 0.1-ml aliquots in microtiter well culture plates, and stimulated with phytohemagglutinin A (PHA) (1 μg/ml) or concanavalin A (ConA) (1 μg/ml) diluted in the culture medium. These doses were found to produce optimal stimulation of lymphocyte proliferation (Pacifici et al., 1999). Nonstimulated cultures were incubated with an equal volume of culture medium. All cultures were incubated at 37 °C for 72 h. After 24 h, 0.2 μCi of methyl-[3H]thymidine, and incubated during 18 h. Cells were harvested in filter paper Skatron 7031 using a Skatron automatic cell harvester. Incorporation of [3H]thymidine was determined by counting in 3 ml of lipolumina scintillation fluid by a beta counter. All cultures were performed in triplicate. Radioactivity was measured in cpm. Results were expressed as stimulation index (SI), defined as the ratio of mean cpm in PHA- or ConA-stimulated versus nonstimulated cultures (expressed as percentage).

**PBMC Stimulation.** PBMC (1 × 10^7 cells/ml) were cultured on 96-well tissue culture plates and stimulated with 2 μg/ml PHA-2 for induction. After 72 h at 37 °C, plates were centrifuged at 800 × g for 10 min, and supernatants were collected and stored at −80 °C (Pacifici et al., 1995). All samples of each subject were assayed in the same analytical batch.

**Cytokine Assays**

Cytokine assays in culture supernatants were performed in 10 subjects due to the extremely high cost of the procedures. The two subjects with lowest amount of supernatant were discarded. There were no statistically significant differences in any subjective/physiological and immunological parameters between subjects undergoing cytokine assays and those who did not. For quantitative measurement of IL-2 and TGFβ, two specific solid phase enzyme amplified sensitivity immunoassays performed on monoclonal antibody-coated microtiter plate were used (Celbio; BioSource, Milan, Italy). For quantitative measurement of IL-10, a specific solid-phase sandwich enzyme-linked immunosorbent assay performed on monoclonal antibody coated microtiter plate was used (Celbio; BioSource). Tests were performed according to the manufacturer’s instructions. Samples and IL standards were assayed simultaneously and in duplicate. The standard curves (assay sensitivity) were as follows: IL-2, 0.9 to 30 (0.1) U/ml; TGFβ, 16 to 2000 (2) pg/ml; and IL-10, 0.78 to 25 (0.21) pg/ml. Cell culture samples were appropriately diluted with the cell culture medium to fit the range of the calibration curves. Assay performance was tested using two concentrations of cytokines in culture medium throughout the procedure. Mean intra-assay and interassay coefficients of variation were <6%.

**Lymphocyte Immunophenotyping**

A total of 100 μl of whole blood was stained using 20 μl of monoclonal antibody reagent. FACS lysing solution (BD Biosciences Italia S.p.A., Milan, Italy) was used to lyse red cells after which stained cells were washed once with phosphate-buffered saline and fixed with 1% paraformaldehyde. Stained and fixed lymphocytes were analyzed using an Ortho Cytoron Absolute 4 flow cytometer (Ortho Instruments, Ortho-Clinical Diagnostic, Milan, Italy). The LeucoGATE (CD45/CD14) fluorescent information, with forward and side scatter, was used to set an electronic gate around the lymphoid population. This gate included at least 95% lymphocytes and less than 5% of other cells (granulocytes, monocytes, and debris). Dual-color immunophenotyping was performed using the following BD Biosciences matched murine monoclonal antibody reagents directly conjugated to phycocerythrine (PE) or fluorescein isothiocyanate (FITC): CD14/PE-C545/CD14 (leucogate reagent for electronic gating), CD4/FITC/HLA-DR/PE (cytotoxic/suppressor cells), CD4/FITC-HLA-DR/PE (helper/inducer cells), CD3/PE-CD16-CD56/FTTC (T mature lymphocytes and natural killer cells), and CD3/PE-CD19/FTTC (T mature lymphocytes and B lymphocytes). The absolute number of lymphocytes was calculated by multiplying the percentage of each lymphocyte subset in the flow cytometer by the absolute lymphocyte count.

**Statistical Analysis**

Values from lymphocyte subsets, functional responsiveness of mitogen-stimulated lymphocytes, plasma cortisol, and prolactin concentrations, and release of cytokines release in supernatants were transformed to differences from baseline and the 9-h area under the curve (AUC) of cortisol and prolactin stimulation kinetics versus time were calculated by the trapezoidal rule. These transformations were analyzed by a two-way repeated measures analysis of variance with the two treatment conditions and time as factors. When treatment condition or the treatment condition × time interaction was statistically significant, multiple Tukey post hoc comparisons were performed at each point of time using the mean square error term of the treatment condition × time interaction. Differences associated with p values lower than 0.05 were considered to be statistically significant.

**Results**

Baseline data on immunological parameters (lymphocyte subsets, functional responsiveness of lymphocytes to mitogenic stimulation, and cytokine production) before drug administration are shown in Table 1. These results are in accordance with permanent alterations in immunological homeostasis found in recreational users of MDMA (Pacifici et al., 2002). The pharmacokinetic interaction between paroxetine and MDMA has been described elsewhere (Parré et al., 2002; Segura et al., 2004). It is worth noticing that MDMA plasma concentrations when coadministered with paroxetine increased modestly by 30% (AUC comparison with the placebo condition), peak concentrations rose by 20%, whereas no changes in T_max were observed. In the same study, a reduced cardiovascular activity and euphoria induced by MDMA were measured in the combination treatment (paroxetine and MDMA).

In the comparison immunological parameter versus exper-
imental day, there were no differences in basal values along the study period in all parameters tested for the 12 participants. Results for treatment conditions and changes over the time in blood lymphocyte subsets, immunoproliferative response to mitogens, plasma cortisol, and prolactin concentrations, and cytokine production by stimulated PBMCs induced by the study drugs are presented in Figs. 1 to 4.

When the single dose of 100 mg of MDMA was administered after 3 days of placebo, alterations of the immune parameters, which peaked at 1.5 h from the start of the MDMA administration, were observed. There was a decrease in circulating T-helper cells (CD4), which showed a maximum mean (S.D.) difference of \(-254.8\ (96.8)\) cell/\(\mu l\) between MDMA and placebo (Fig. 1). CD3 T cells showed a mean maximum decrease of \(-182.2\ (73.1)\) cells/\(\mu l\) from baseline (Fig. 1). Lymphoproliferative response to PHA and ConA stimulation was also reduced to a maximum of \(-37.3\ (16.1)\%\) and \(-25.0\ (10.2)\%\), respectively (Fig. 2). No differences were found in the amount of cytotoxic/suppressor lymphocytes (CD8) (Fig. 1). In contrast, there was a high increase in NK cells with a mean peak of 59.5 (39.9) cells/\(\mu l\) (Fig. 2). MDMA treatment was also associated with a decrease in the production of the Th1-type cytokine IL-2 (peak effect of \(-8.7\) U/ml) and an increase in the production of the Th2-type cytokine IL-10 (peak effect of 1809.3 pg/ml) (Fig. 3). The anti-inflammatory cytokine TGF\(\beta_1\) presented a peak increase difference induced by MDMA of 1877.6 pg/ml (Fig. 3). Finally, MDMA produced a mean rise in cortisol and prolactin concentrations at 2 h after drug administration of 21.8 \(\mu g/dl\) and 24.6 ng/ml, respectively (Fig. 4).

Administration of paroxetine for three consecutive days did not produce any significant alteration in cell-mediated immune responses nor in the release of cytokines. However, when MDMA was administered after paroxetine pretreatment, the observed immunological changes were less evident than those induced in the placebo/MDMA condition. Indeed,

<table>
<thead>
<tr>
<th>Lymphocyte Subsets</th>
<th>Mean (S.D.)</th>
<th>Cytokine Production</th>
<th>Mean (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4</td>
<td>cell/(\mu l)</td>
<td>761.7 (84.3)</td>
<td>IL-2</td>
</tr>
<tr>
<td>CD8</td>
<td>cell/(\mu l)</td>
<td>494.6 (87.2)</td>
<td>IL-10</td>
</tr>
<tr>
<td>CD4/CD8 Ratio</td>
<td>1.6 (0.5)</td>
<td></td>
<td>TGF(\beta_1)</td>
</tr>
<tr>
<td>CD3</td>
<td>cell/(\mu l)</td>
<td>1392.6 (92.0)</td>
<td></td>
</tr>
<tr>
<td>NK</td>
<td>cell/(\mu l)</td>
<td>54.6 (31.0)</td>
<td></td>
</tr>
<tr>
<td>CD19</td>
<td>cell/(\mu l)</td>
<td>199.2 (60.2)</td>
<td></td>
</tr>
<tr>
<td>S / I PHA</td>
<td>%</td>
<td>86.0 (9.1)</td>
<td></td>
</tr>
<tr>
<td>S / I ConA</td>
<td>%</td>
<td>52.2 (13.5)</td>
<td></td>
</tr>
<tr>
<td>CD19 S / I PHA</td>
<td>%</td>
<td>86.0 (9.1)</td>
<td></td>
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<tr>
<td>CD19 S / I ConA</td>
<td>%</td>
<td>52.2 (13.5)</td>
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**TABLE 1**
Basal values (mean and S.D.) of lymphocyte subpopulations (n = 12) and cytokine production (n = 10) in healthy participants

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![Image](https://via.placeholder.com/150)

**Fig. 1.** Time course (0–72 h) of drugs effects on CD4, CD8, and CD3 T cells (differences from baseline). Data points represent means ± S.E.M. from 12 subjects. ○, placebo at days 1, 2, and 3 and MDMA (100 mg) at day 3; □, paroxetine (20 mg/day) at days 1, 2, and 3 and MDMA 100 mg at day 3 (MDMA administration is indicated by an arrow). At each time, filled symbols indicate a significant difference from placebo (black fill, \(p < 0.01\)).
Paroxetine pretreatment reduced by 33% the MDMA effects on suppression in the CD4 T-cells [mean (S.D.)] peak of –157.5 (67.3) cells/μl with a consequent similar suppression of the CD3 T cells (Fig. 1). A significant 50% reduction between MDMA alone and preceded by paroxetine administration was also observed in case of NK cells increase, which reached a mean plateau of 29.5 (14.8) cells/μl (Fig. 2). The decrease in lymphoproliferative response to PHA and ConA stimulation induced by MDMA was completely inhibited by paroxetine pretreatment (~3.9 and ~2.0% maximum decrease with respect to baseline) (Fig. 2).

The same effects were noted in MDMA-induced alteration of cytokine release by PBMCs. A significant 67% attenuation of the decrease in IL-2 production (mean peak ~2.6 U/ml) and more than 90% reduction in IL-10 and TGFβ, MDMA-induced increase (mean maximum increase of 99.5 and 123.1 pg/ml, respectively) were observed after paroxetine pretreatment (Fig. 3). Paroxetine decreased to approximately one-half MDMA-induced stimulation of cortisol and prolactin (peak effect 13.1 μg/dl and 10.1 ng/ml, respectively) with a statistical significant reduction in the areas under the curve (AUC0–9h) of the two hormones compared with the placebo/MDMA condition (cortisol 1627.4 versus 1277.0 μg/dl·h, prolactin 1335.6 versus 1013.9 ng/ml·h) (Fig. 4).

**Discussion**

The main finding of this study is that MDMA-induced immune dysfunction was mostly counteracted by paroxetine, despite a 30% increase in MDMA plasma concentration resulting from the metabolic interaction of paroxetine and MDMA (Farré et al., 2002; Segura et al., 2004).

As already assessed in several clinical trials (Pacifici et al., 1999, 2000, 2001a,b, 2002) with MDMA consumers, a single MDMA dose induced a redistribution of lymphocytes with a decrease in circulating T-helper cells (CD4) and a simulta-
neous increase in NK cells. This represented an immunosuppressive action (cells were removed from primary site of action with a reduction of cytotoxic activity; Friedman and Irwin, 1997), and in fact a decrease in the functional responsiveness of lymphocytes to mitogenic stimulation was observed. Furthermore, IL-2, one of key cytokines involved in stimulation of lymphocytes proliferation, was suppressed by MDMA administration (Pacifici et al., 2001a,b). Conversely, IL-10 and TGFβ1, pleiotropic immunosuppressive and anti-inflammatory cytokines involved in negative immunoregulatory functions, such as inhibition of the proliferative response and cytokine production by T-helper cells, were stimulated (Mosmann, 1994; Sosroseno and Herminajeng, 1995). These changes in immune function were putatively linked to increased cortisol and prolactin concentration, which were in turn a consequence of the effects of MDMA administration on central monoaminergic systems with the release of corticotrophin-releasing factor and subsequent activation of the hypothalamus-pituitary axis (Grob et al., 1996).

Alterations in peripheral blood lymphocytes redistribution and stimulation of cortisol and prolactin kinetics were only partially blunted by paroxetine, whereas the suppressive effects on lymphocytes functionality and modulation of cytokine release were completely reversed.

To hypothesize the effect of paroxetine pretreatment on acute effects of MDMA on cell-mediated immune response in humans, it has to be recalled that MDMA affects a variety of neurotransmission systems, which in turn regulate the immune system activity. As previously mentioned, MDMA mainly releases presynaptic 5-HT, reversing the 5-HT uptake transporter (Vollenweider et al., 2002). Hence, large amounts of serotonin are released in the central nervous system and peripheral tissues, including the constituents of the immune system. Indeed, serotonergic receptors and serotonin transporters have been characterized on immune cells (Eliseeva and Stefanovich, 1982; Marazziti el al., 1998).

The role of serotonin in the immune system has been extensively reviewed (Mössner and Lesch, 1998). Major functions that emerge for 5-HT are NK cell activation and inhibition of T cell proliferation after mitogen stimulation (PHA and ConA), involving 5-HT-1A and 5-HT-2 receptors, respectively (Mizruchin et al., 1999). In agreement with that evidence, Mizruchin et al. (1999) recently suggested that cytolytic activity of NK cells is regulated by 5-HT-1A receptors. The effects observed in human subjects after the administration of a serotonergic challenge, such as MDMA, are in agreement with the role that serotonin seems to play in the regulation of the immune system (Pacifici et al., 1999, 2000, 2001a,b). A SSRI pretreatment should affect all those functions regulated by serotonin in the immune system once challenged by a serotonergic drug. In fact, the SSRI paroxetine showed a very high affinity for the binding site at the serotonin transporter characterized in human resting lymphocytes (Marazziti et al., 1998).

Paroxetine binds competitively to the 5-HT uptake site and antagonizes MDMA activity, either preventing its interaction with the 5-HT uptake site or alternatively blocking the efflux of 5-HT through the carrier. In this respect, paroxetine could
inhibit completely presynaptic 5-HT release caused by MDMA administration in humans. The present results strongly suggest that the MDMA-induced inhibition of the lymphoproliferative response to mitogens as well as the altered release of cytokines such as IL-2, IL-10, and TGFβ1 is mainly serotonin-driven. Nevertheless, several alterations on the immune system and in the release of hormones after MDMA administration seem to be regulated by mechanisms other than the serotonergic system. Paroxetine as CYP2D6 inhibitor altered MDMA pharmacokinetics and an increase in drug concentrations (20% $C_{\text{max}}$ and 30% AUC) in humans have been observed (Farré et al., 2002; Segura et al., 2004). Higher MDMA concentration cannot interfere with serotonin release because the transporter is blocked by paroxetine, but it can stimulate other receptors, including postsynaptic 5-HT-2, alpha-2-adrenergic, M-1 cholinergic, trace amines (Bunzow et al., 2001), and H-1 histamine receptors (Vollenweider et al., 2002) or inhibit the uptake of other neurotransmitters, such as norepinephrine (Rothman et al., 2001). An interesting effect resulting from immunological dysfunction, is the MDMA induction of dopamine (DA) release directly by reversal of the DA uptake carrier and secondarily through the activation of postsynaptic 5-HT-2A receptors (Bogen et al., 2003). DA is known to play a role in the immunological surveillance, with no definitive effect on stimulation or inhibition of functional activities of different immunocompetent cells (Basu and Dasgupta, 2000). Then, paroxetine would be expected to have no effect on MDMA-induced carrier mediated DA release and its manifestations.

Furthermore, as a consequence of the paroxetine-mediated metabolic inhibition of MDMA, a decrease in the recoveries of the two main MDMA metabolites: 4-hydroxy-3-methoxymethamphetamine (HMMA) and 3,4-dihydroxymethamphetamine was observed (Segura et al., 2004). In vitro data suggest that HMMA may contribute significantly to the secretion of arginine-vasopressin (Forsling et al., 2001; Fallon et al., 2002) observed after MDMA administration (Henry et al., 1998). Arginine-vasopressin secretion has been associated with hyponatraemia observed in some MDMA consumers (Forsling et al., 2001; Fallon et al., 2002). Vasopressin has been found to be involved in the suppression of proliferative response of splenic T cells and NK cytotoxicity in animal models (Shibasaki et al., 1998), and a contribution from this hormone to the immune modulation of MDMA in humans may be hypothesized. A reduction in HMMA disposition due to paroxetine pretreatment may determine a possible reduction in vasopressin secretion with consequent reduction in its immunosuppressive actions. The role of MDMA metabolites on the immune system deserves further studies.

In relation to neuroendocrine effects, MDMA produced an increase in plasma cortisol and prolactin concentrations, as reported previously (Mas et al., 1999; Hernandez-Lopez et al., 2002). As a result of the activity of these hormones, it may be speculated that subsequent inhibition of several lymphocyte functions and alterations in cytokines release (Matera, 1996; Wilckens and De Rijk, 1997). Although cortisol release is consistent with activation of serotonergic neurotransmission, dopaminergic and noradrenergic mechanisms have also been postulated (Mas et al., 1999). Similarly, prolactin secretion is mainly mediated by both dopaminergic and serotoninergic systems. These findings are consistent with only partial reduction by paroxetine of MDMA-induced neuroendocrine stimulation.

In summary, several receptors and biological interactions may be involved in mediating the immunomodulating properties of MDMA. The release of 5-HT is an important MDMA action, which is almost completely inhibited by paroxetine binding to the 5-HT uptake site or 5-HT transporter blocking. Consequently, it may be hypothesized that MDMA-induced suppression of functionality of lymphocytes, expressed as lymphoproliferative response of PBMCs to mitogenic stimulation and modulation of PBMC cytokine release, is only mediated by 5-HT. By contrast, lymphocyte subsets redistribution induced by MDMA, similarly to cortisol and prolactin kinetics stimulation, may support the participation of postsynaptic 5-HT-2 and DA receptors as well as vasopressin and trace amines, although the role played by neurotransmitters other than 5-HT in mediating the immunomodulating effects of MDMA remains to be determined. Further investigations in humans using specific receptor ligands are warranted, particularly for assessing the contribution of postsynaptic 5HT-2, DA, and norepinephrine as well as vasopressin and trace amines in MDMA-mediated immune effects.

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Fig. 4. Time course (0–9 h at the third administration day) of drugs effects on plasma cortisol and prolactin concentration (differences from baseline). Data points represent means ± S.E.M. from 12 subjects. □, placebo at days 1, 2, and 3 and MDMA (100 mg) at day 3; ◆, paroxetine (20 mg/day) at days 1, 2, and 3 and MDMA (100 mg) at day 3 (MDMA administration is indicated by an arrow). At each time, filled symbols indicate a significant difference from placebo (gray fill, $p<0.05$; black fill, $p<0.01$).


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